abcam

Product datasheet

Human RARA (Retinoic Acid Receptor alpha) knockout HeLa cell line ab265176

2 Images

Overview

Product name	Human RARA (Retinoic Acid Receptor alpha) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 7
Passage number	<20
Knockout validation	Sanger Sequencing
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HeLa cell line (**ab255928**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Receptor for retinoic acid. Retinoic acid receptors bind as heterodimers to their target response elements in response to their ligands, all-trans or 9-cis retinoic acid, and regulate gene expression in various biological processes. The RXR/RAR heterodimers bind to the retinoic acid response elements (RARE) composed of tandem 5'-AGGTCA-3' sites known as DR1-DR5. In the absence of ligand, the RXR-RAR heterodimers associate with a multiprotein complex containing transcription corepressors that induce histone acetylation, chromatin condensation and transcriptional suppression. On ligand binding, the corepressors dissociate from the receptors and associate with the coactivators leading to transcriptional activation. RARA plays an essential role in the regulation of retinoic acid-induced germ cell development during spermatogenesis. Has a role in the survival of early spermatocytes at the beginning prophase of meiosis. In Sertoli cells, may promote the survival and development of early meiotic prophase spermatocytes. In concert with RARG, required for skeletal growth, matrix homeostasis and growth plate function (By similarity). Regulates expression of target genes in a ligand-dependent manner by recruiting chromatin complexes containing MLL5. Mediates retinoic acid-induced granulopoiesis.
Involvement in disease	Note=Chromosomal aberrations involving RARA are commonly found in acute promyelocytic leukemia. Translocation t(11;17)(q32;q21) with ZBTB16/PLZF; translocation t(15;17)(q21;q21) with PML; translocation t(5;17)(q32;q11) with NPM. The PML-RARA oncoprotein requires both the PML ring structure and coiled-coil domain for both interaction with UBE2I, nuclear microspeckle location and sumoylation. In addition, the coiled-coil domain functions in blocking RA-mediated transactivation and cell differentiation.

Sequence similarities	Belongs to the nuclear hormone receptor family. NR1 subfamily. Contains 1 nuclear receptor DNA-binding domain.
Domain	Composed of three domains: a modulating N-terminal domain, a DNA-binding domain and a C-terminal ligand-binding domain.
Post-translational modifications	Phosphorylated on serine and threonine residues. Phosphorylation does not change during cell cycle. Phosphorylation on Ser-77 is crucial for transcriptional activity (By similarity). Phosphorylation by AKT1 is required for the repressor activity but has no effect on DNA binding, protein stability nor subcellular localization. Phosporylated by PKA in vitro. This phosphorylation on Ser-219 and Ser-369 is critical for ligand binding, nuclear localization and transcriptional activity in response to FSH signaling. Sumoylated by SUMO2, mainly on Lys-399 which is also required for SENP6 binding. On all-trans retinoic acid (ATRA) binding, a confromational change may occur that allows sumoylation on two additional site, Lys-166 and Lys-171. Probably desumoylated by SENP6. Sumoylation levels determine nuclear localization and regulate ATRA-mediated transcriptional activity. Trimethylation enhances heterodimerization with RXRA and positively modulates the transcriptional activation. Ubiquitinated.
Cellular localization	Nucleus. Cytoplasm. Nuclear localization depends on ligand binding, phosphorylation and sumoylation. Transloaction to the nucleus in the absence of ligand is dependent on activation of PKC and the downstream MAPK phosphorylation.

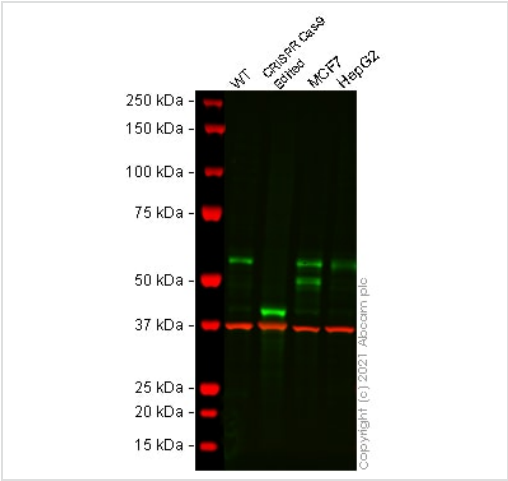
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265176 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 51 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

Images



Western blot - Human RARA (Retinoic Acid Receptor alpha) knockout HeLa cell line (ab265176)

All lanes : Anti-Retinoic Acid Receptor alpha antibody [EPR23871-271] (**ab275745**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RARA CRISPR-Cas9 edited HeLa cell lysate
Lane 3 : MCF7 cell lysate
Lane 4 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 51 kDa
Observed band size: 40 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab275745** observed at 40 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab275745 was shown to react with Retinoic Acid Receptor alpha in wild-type HeLa cells in western blot. The bands observed in RARA knockout cell line ab265176 (RARA knockout cell lysate **ab257629**) below 40 kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and RARA CRISPR/Cas9 HeLa edited cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab275745** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

Sanger Sequencing - Human RARA knockout HeLa cell line (ab265176)

Homozygous: 1 bp insertion in exon 7.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors